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(12) # EUROPEAN PATENT APPLICATION

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(54) **Novel organism and use thereof in production of functionalized whey products.**

(57) A biologically pure culture of the organism *Xanthomonas campestris* ATCC 31923 is disclosed.

A process for the production of a functionalized dairy whey product characterised in that it comprises:

(a) providing a fermentation broth of whey and yeast extract; and

(b) fermenting the broth with the organism *Xanthomonas campestris* ATCC 31923 to produce a functionalized dairy whey product containing a thickening polymer produced by the organism;

and, optionally,

(c) drying the functionalized whey product to form a dry functionalized whey product is also disclosed.

Dairy whey, a waste product of cheese production, may be functionalized by fermentation techniques to produce a functionalized whey which serves as a thickening agent in the food industry. This simultaneously provides a method for utilizing the whey waste produced.

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"NOVEL ORGANISM AND USE THEREOF IN PRODUCTION OF FUNCTIONALIZED WHEY PRODUCTS"

This invention relates to a novel organism and to the use thereof in the production of functionalized whey products; more particularly, it relates to a method for functionalizing whey by forming a fermentation broth of the whey and yeast extract and then fermenting this whey broth with the novel organism Xanthomonas campestris BB-1L (ATCC 31923).

There are no cellular or colonial morphological differences between the deposited culture ATCC 31923 and the parent, Xanthomonas campestris. There are physiological characteristic differences which are referred to herein. Reference may be made to Bergey's Manual of Derterminative Bacteriology, 8th Edn., 243-245, for colonial morphological, cellular and physiological characteristics of the genus Xanthomonas and the species Xanthomonas campestris. Reference may also be made to the related copending application No. (SS/CF 3661).

Controlled fermentation of foods can be used as a means of improving functionality of the foods. Dairy whey, a food, may be an economical source of a fermentable substrate, and is widely used as an accepted milk-derived ingredient in manufactured foods. If whey can be properly functionalized by fermentation with an organism that produces a thickening polymer when grown on the whey substrate, it is possible to obtain whey products that may serve the function of a stabilizer, thickener, emulsifier, or flavor enhancer.

Whey is the fluid medium containing a very low concentration of milk solids and a high concentration of lactose. Disposal of this waste by-product by drying is an

energy-intensive, expensive procedure which results in an expensive by-product, while sewering of the whey is prohibitive in cost due to the high biological oxygen demand which is placed on municipal sewer systems.

The most desirable method of handling this waste stream is to produce a high quality natural food ingredient from the whey waste product. A novel method of producing a functionalized whey product for use as a food ingredient or any type of product where milk solids and lactose are acceptable ingredients has now been discovered.

Description of the Drawings

Figure I shows a graph of a typical fermentation of Xanthomonas campestris ATCC 31923 in a medium containing 4% Teklack (whey), and 0.05% yeast extract.

Figure II shows a viscosity versus shear rate curve for a typical dried functionalized whey produced by the fermentation techniques of this invention.

Figure III shows a graph of a regression analysis of viscosity v. number of generations ATCC 31923 was grown in whey medium.

Detailed Description of the Invention

A functionalized dairy whey product having a viscosity greater than 200 centipoise at a $12s^{-1}$ sheer rate for use as a food ingredient that may serve as a stabilizer, thickener, or emulsifier, can be produced by fermenting a mixture comprising whey, optionally yeast extract and a pH buffer with the novel organism Xanthomonas campestris ATCC 31923 to produce a functionalized whey product containing a thickening polymer produced by the novel organism Xanthomonas campestris ATCC 31923.

Derivation of Xanthomonas campestris ATCC 31923

X. campestris ATCC 31923 was isolated for its ability to grow on lactose as sole source of carbon and energy, It was derived from X. campestris BB-1 (ATCC 31922) following several serial passages in lactose minimal medium containing 1.5% lactose, 0.5% K_2HPO_4, 0.2% NH_4Cl, 0.1% NaCl, 0.01% $MgSO_4$, and 0.01% yeast extract. In this medium, at about 28°C, ATCC 31923 has a generation time of about three hours, viable cell titers of about 10^9/ml or greater are reached, the lactose in the medium is metabolized, and the broth does not become viscous.

When X. campestris ATCC 31923 was subsequently grown in whey medium containing 2% Teklac, 0.25% K_2HPO_4, 0.01% yeast extract at about 28°C, the generation time was about three hours, viable titers, of about 10^9 cells/ml or greater were reached, the lactose in the medium was metabolized, and the broth became viscous.

Although it is known in the art that an ultra-filtered and hydrolyzed whey medium fermented with Xanthomonas campestris results in excellent polymer formation, all growth to date on unhydrolyzed whey has

failed to result in polymer production; see, K.R. Stauffer and J.G. Leeder, 1978, J. Food Sci., 43: 756-758, "Extracellular Microbial Polysaccharide Production by Fermentation on Whey or Hyrolyzed Whey," and M. Charles and M.K. Radjai, 1977 "Xanthan Gum From Acid Whey" in Extracullular Microbial Polysaccharides, eds. P.A. Sandford and A.I. Laskin. ACS Symp. Ser. No. 45, pp. 27-39. Fermentation using ATCC 31923 of a whey broth comprising unhdyrolyzed whey (acid or sweet), and optionally yeast extract results in polymer formation and functionalization of the whey so that the whey product can be utilized as a food ingredient. This aerobic fermentation can be carried out preferably in a pH range of 6 to 8, preferably with the pH maintained in a range from about 6.5 to about 7.5. The fermentation can be carried out at a temperature from about 20 to 35°C, preferably carried out at a temperature from about 25 to about 30°C. Typical composition of Teklac (sweet dairy whey) is as follows:

CHEMICAL AND PHYSICAL SPECIFICATIONS

Ingredient
Listing: Whey

Typical Proximate Analysis	
Protein (N x 6.38)%	12.7
Fat %	1.1 (1.25% Maximum)
Moisture %	4.5 (5.0% Maximum)
Ash %	8.0
Lactose %	71.3
Calories, Cal/100g	350.0

Typical Vitamin & Mineral Analysis	
Vitamin A I.U./100g	Nil
Vitamin C mg/100g	Nil
Thiamin mg/100g	0.40
Riboflavin mg/100g	1.76
Niacin mg/100g	1.00
Calcium %	0.71
Iron %	Nil
Vitamin B_{12} ug/100g	2.12

Typical Vitamin & Mineral Analysis (continued)	
Phosphorus %	0.69
Pantothenic Acid mg/100g	4.09

Microbiological Standards	
Standard Plate Count	10,000/g (Maximum)
Coliforms	9/g (Maximum)
E. coli	Negative
Salmonella	Negative

The nutritional values listed above are within 80% of the value declared in compliance with Federal Nutritional Regulations 21 CFR §1.17(4)(ii).

	Typical Range	Limit
Solubility Index	0.1 - 0.5 ml	1.25 ml Max.
Acidity	0.10 - 0.14%	0.16 Max.
Alkalinity of Ash	175 - 200 ml	225 ml Max.
Scorched Particles	7.5 mg	15.0 mg Max.
Particle size (Through 40 Mesh)	99 - 100%	98% Min.

Concentration of whey can range from about 0.5% to about 12.0%, preferably 2% to 4%. The additional yeast extract in the fermentation broth can range from about 0 to about 0.5%, preferably from about 0.01% to about 0.1%. Adequate fermentation broth viscosities (>200 cps and preferably >800 cps at a 12 s^{-1} shear rate) are usually reached within 48 to 72 hours. All of the above weight percents are in weight per volume.

X. campestris ATCC 31923 was isolated by continuous enrichment and selection in a lactose minimal medium from the parent strain, ATCC 31922, which either grows poorly or not at all, and produces little or no polymer, when lactose is the sole source of carbon and energy. Further, ATCC 31922 grows well but does not produce polymer on whey medium without glucose supplementation, and the lactose in the whey is not used.

To ensure the ability of ATCC 31923 to grow and produce polymer in whey medium the strain is routinely maintained in lactose minimal medium during storage and inocula production. When polymer production is desired a lactose minimal medium grown culture is transferred to whey medium. Prolonged maintenance in whey results in the loss of the ability of ATCC 31923 to produce viscous broths in whey indicating a reversion to preferential growth on protein.

EXAMPLE 1

Figure 1 shows a graph of a typical fermentation of Xanthomonas campestris ATCC 31923 in a medium containing 4.0% Teklac and 0.05% yeast extract. The medium was sterilized by autoclaving at15 pounds per square inch (psi) for 15 minutes. The fermentation was conducted in a fermentor to which air was pumped at the rate of 1 volume/volume/min, agitation was at the rate of 500 rpm, and the dissolved oxygen concentration maintained at a minimum of 20% saturation. A Bio-flow® fermentor was used (New Brunswick Scientific Co., N.J.). The initial pH was about 6.5 and was controlled between 6.5 and 7.5. The inoculum was 3% volume/volume from a lactose minimal medium grown culture. The figure shows the general increase in viscosity over time, growth of the organism, and the initial increase in pH, followed by a decrease in pH, typical of this fermentation, and a decrease in lactose concentration.

The high viscosity broths produced by fermentation techniques of this invention may be dried and/or sterilized by autoclave plus lyophilization, spray drying, or other techniques.

EXAMPLE 2

A viscosity versus shear rate curve for a typical dried functionalized whey so produced is shown in Figure II. The sample was tested on a 2.5 XLVT Wells-Brookfield microviscometer having a 3° cone at 25°C. The sample size was 2.0 milliliters. The sample consisted of a 1% solution (weight/vol) of functionalized whey in deionized water. The pH was 7.0 and lactose concentration was 2.6 grams per liter. The increase in viscosity with decrease in shear rate is typical of pseudoplastic polymers.

EXAMPLE 3

Prolonged maintenance in whey results in the loss of the ability of ATCC 31923 to induce viscous broths in whey. ATCC 31923 was serially transferred (2% vol/vol) at 48 hour intervals, in Teklac medium, for a total of 85 generations. The medium contained 2% Teklac, 0.25% K_2HPO_4, 0.1% yeast extract. At the time of transfer the viscosity was measured and the culture titered on YM agar (Difco, Detroit, Michigan).

The results are shown in Table I. For the first 35 generations (5 transfers) the broth viscosity remained high at about 400 cps. For the next 45 generations (8 tranfers) the viscosity dropped to 100-200 cps. A regression analysis of the viscosity v generation number is shown in Figure III. The reversion frequency (loss of ability to produce high viscosity broths in Teklac medium) is such that for at least about 55 generations broths with viscosities >200 cps are produced. However, continued transfer in whey eventually resulted in loss of the abiity to produce viscous broths.

TABLE I

X. Campestris ATCC 31923 Stability. Growth and polymer production in 2% Teklac, 0.25% K_2HPO_4, 0.1% yeast extract.

Transfer Number	Titers, cfu/ml		Generations		Viscosity cps @ 12 s⁻ @48h
	0 hours	48 hours	per transfer	cumulative	
1	1.8×10^7	2.6×10^9	7.2	7.2	453
2	3.5×10^7	2.5×10^9	6.2	13.4	398
3	1.0×10^7	4.0×10^7	2.0	15.4	402
4	2.0×10^5	7.9×10^8	11.9	27.3	>480
5	1.4×10^7	3.0×10^9	7.8	35.1	378
6	6.0×10^7	2.1×10^9	5.1	40.2	129
7	4.2×10^7(cal)	1.0×10^9	4.6	44.8	206
8	1.2×10^7	1.0×10^9	6.4	51.2	208
9	1.5×10^7	1.0×10^9	6.1	57.3	161
10	1.9×10^7	4.7×10^7	1.3	58.6	158
11	1.0×10^5	1.3×10^8	10.4	69.0	91
12	2.6×10^6(cal)	1.1×10^9	8.8	77.8	95
13	3.7×10^7	2.0×10^8	2.4	80.2	129
14	6.0×16^6(cal)	3.0×10^8	5.6	85.8	58

cfu/ml = colony forming units/ml.

The functionalized whey product of this invention can be used as a food ingredient where milk solids and/or whey, and/or thickeners, and/or stabilizers are used such as in ice cream, salad dressing, foam stabilizer (meringue), puddings, snack foods, etc.

CLAIMS:

1. A biologically pure culture of the organsim Xanthomonas campestris ATCC 31923.

2. A process for the production of a functionalized dairy whey product characterised in that it comprises:

(a) providing a fermentation broth of whey and yeast extract; and

(b) fermenting the broth with the organism Xanthomonas campestris ATCC 31923 to produce a functionalized dairy whey product containing a thickening polymer produced by the organism; and, optionally,

(c) drying the functionalized whey product to form a dry functionalized whey product.

3. A process as claimed in claim 2 wherein the concentration of the whey is from 0.5 to 12% weight per volume and the concentration of the yeast extract is from 0 to 0.5% weight per volume.

4. A process as claimed in claim 3 wherein the concentration of the yeast extract is from 0.01 to 0.1% weight per volume.

5. A process as claimed in any of claims 2 to 4 wherein the fermentation is conducted at a temperature of from 20 to 35°C.

6. A process as claimed in any of claims 2 to 5 wherein the fermentation is conducted at a pH of from 6 to 8.

FIGURE I

X. CAMPESTRIS ATCC-31923 FERMENTATION IN WHEY MEDIUM

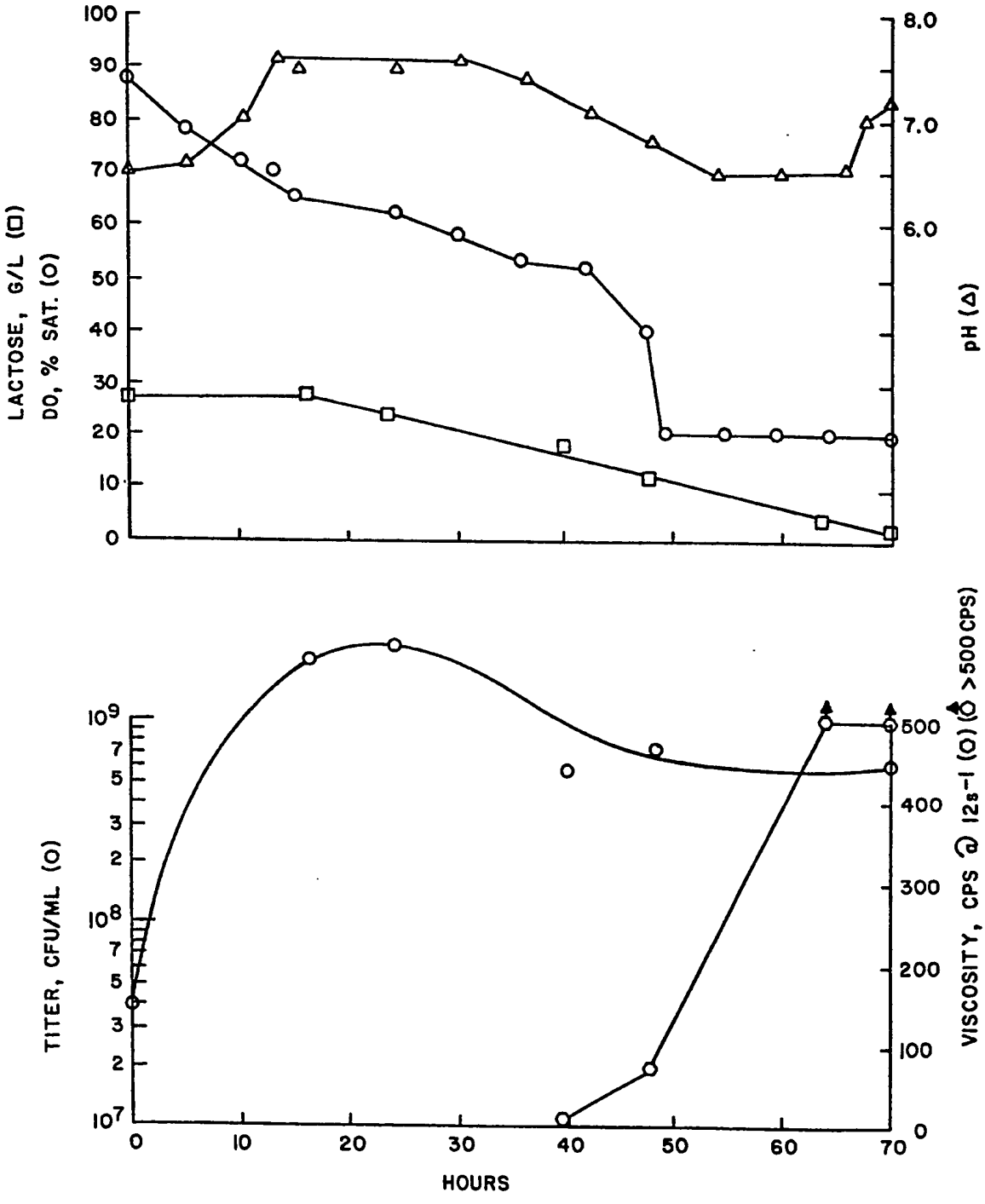


FIGURE II

VISCOSITY VS. SHEAR RATE CURVE FOR DRIED FUNCTIONALIZED WHEY

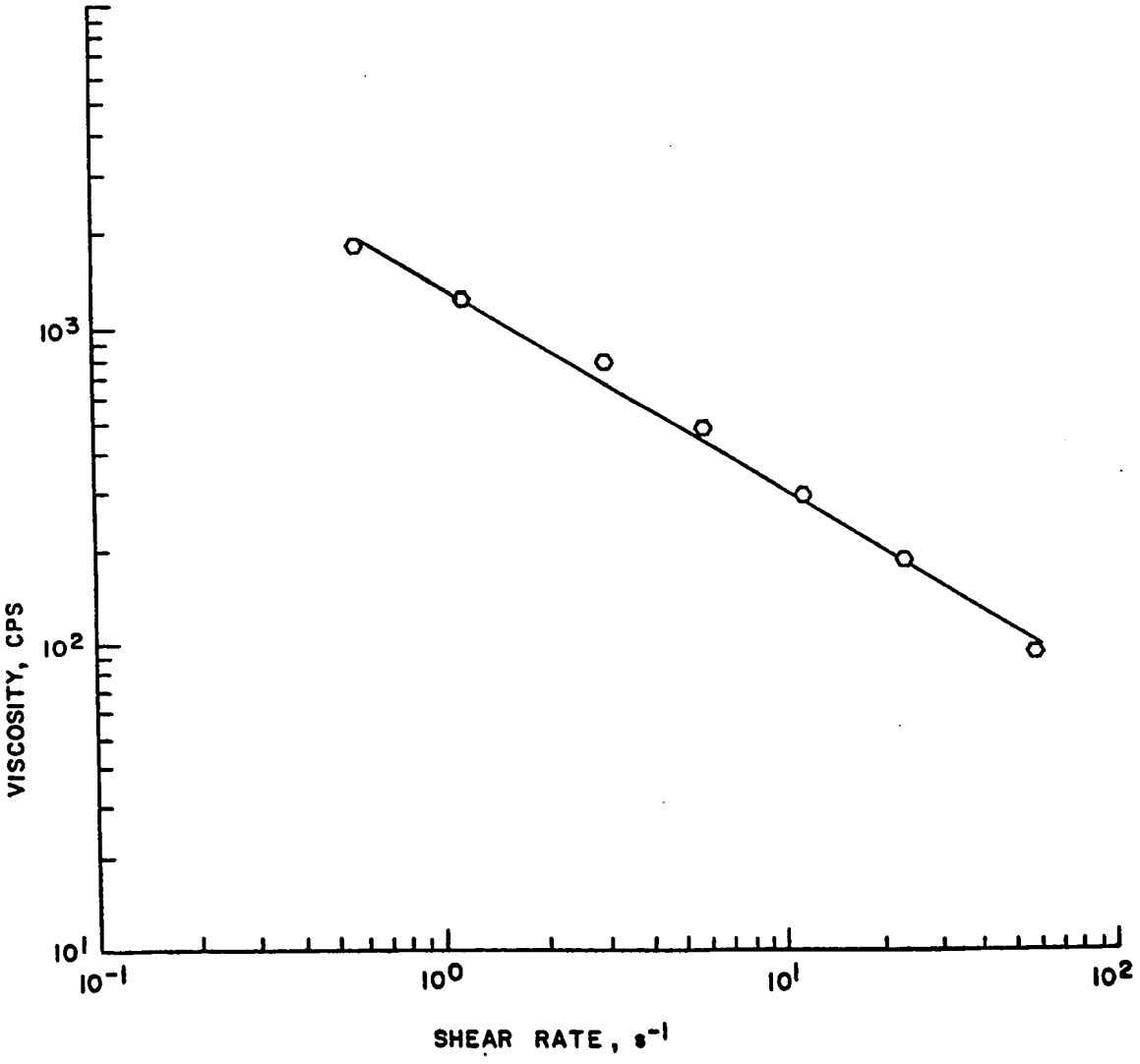
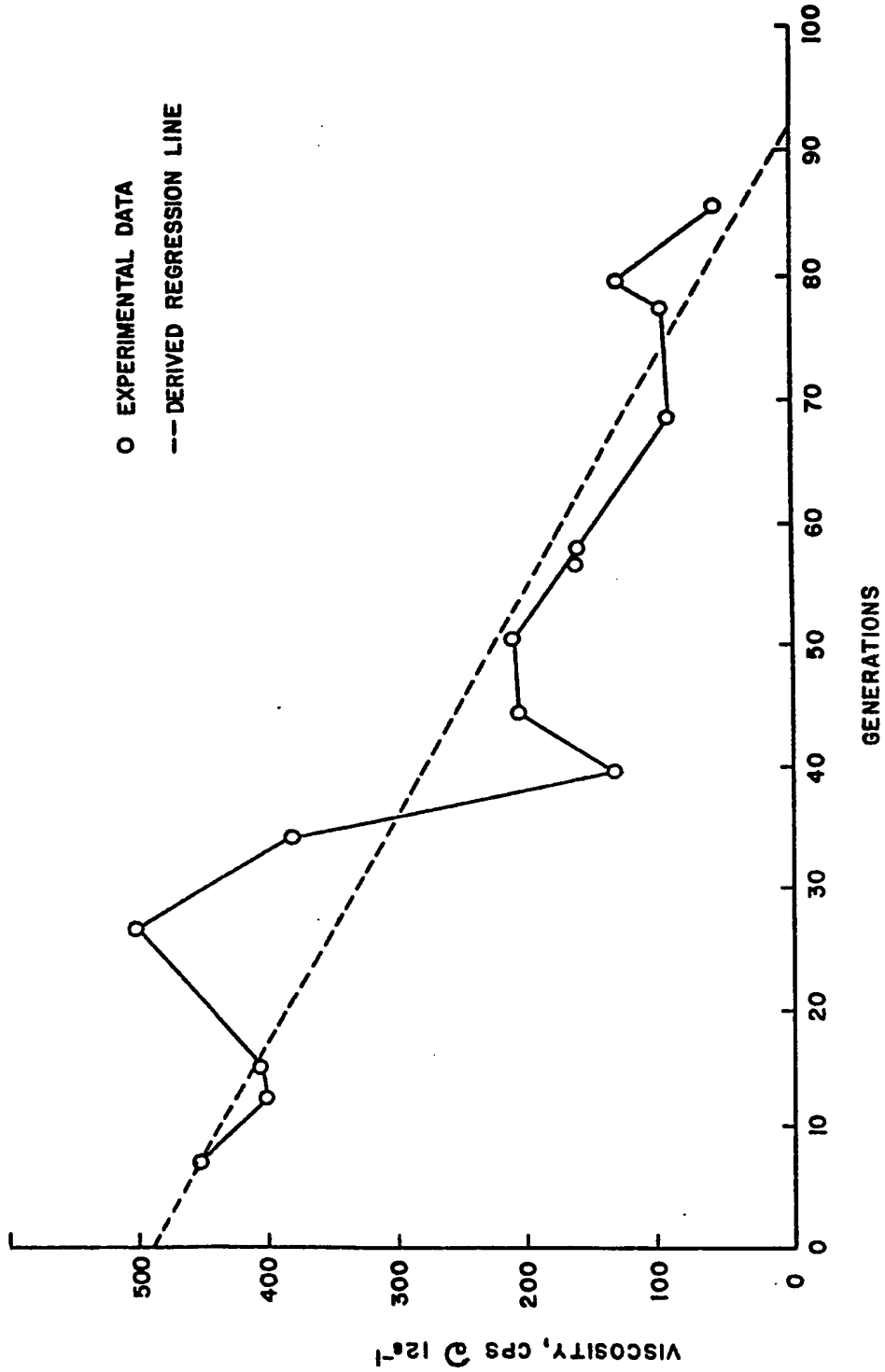


FIGURE III

REGRESSION ANALYSIS OF VISCOSITY VS. NO. GENERATIONS ATCC-31923 IN TEKLAC MEDIUM



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EUROPEAN SEARCH REPORT

0071380

Application number

EP 82 30 3775

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
D,X	JOURNAL OF FOOD SCIENCE, vol. 43, 1978, pages 756-758; K.R.STAUFFER et al.: "Extracellular microbial polysaccharide production by fermentation on whey or hydrolyzed whey". *Page 757*	1	C 12 P 19/06 A 23 C 21/02
D,X	EXTRACELLULAR MICROBIAL POLYSACCHARIDES, Editors P.Sandford and A.Laskin, American Chemical Society Symposium Series, no. 45, 1977, pages 27-39; M.CHARLES et al.: "Xanthan gum from acid whey". *The whole document*	1	
X	FR-A-2 442 888 (PFIZER) *Claims 1-4; examples 1 and 2*	1	TECHNICAL FIELDS SEARCHED (Int. Cl. ³)
A	FR-A-1 526 105 (LES USINES DE MELLE) *Abstract*	1,2	C 12 P 19/00 A 23 C 21/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	27-10-1982	DELANGHE L.L.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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